

IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

Applicant: CLEARY, et al.  
Serial No.: 10/529,622  
Filed: March 30, 2005  
For: Highly Purified Amphotericin B  
Group Art Unit: 1623  
Examiner: PRESELEV, Eli  
Attorney's Docket No. 11636N/020724  
Customer No. 32885

SECOND DECLARATION UNDER 37 C.F.R. § 1.132

Commissioner for Patents  
P.O. Box 1450  
Alexandria, VA 22313-1450

Dear Sir:

I, Robert E. Kramer, Ph.D., declare:

1. I have significant experience in the field of pharmacology. My current position is Professor, Department of Pharmacology and Toxicology, University of Mississippi Medical Center. I consider myself to be one of ordinary skill in the art.

2. I have previously submitted a Declaration under 37 C.F.R. § 1.132 in connection with the above-identified patent application.

3. I have read and understand the Office Action mailed August 12, 2008, in connection with the above-identified application. Furthermore, I have read and understand the patents cited therein.

4. The Office Action mailed August 12, 2008 states that "applicant has not provided any evidence in verified form that amphotericin B produced by the purification methods of the cited prior art would not result in a product having purity greater than 89%." See page 4 of the outstanding Office Action.

5. As stated in a previous Declaration, Michel et al., US '789, relied upon by the outstanding Office Action in connection with purification issues, discloses a four solvent system for purifying amphotericin B: methanol, dimethylformamide, methylene chloride, and water. In US '789, purity is measured as "residue on ignition." Even an implied purity of 99.9% based on residue after ignition ( $\leq 0.1\%$ ) is not a valid or appropriate measurement in terms of the claimed compositions (comprising at least 96% amphotericin B and 4% or less of impurity products). Ignition *per se* is not a quantitative means of estimating purity. If the 'ignition test' was done properly, any organic compound consisting of carbon, hydrogen and nitrogen – e.g., amphotericin B and other polyene contaminants – would be expected by one in the field to ignite completely. Thus, under proper conditions, the residue remaining after ignition is an index of inorganic or metal content and not of

purity of the organic material. One of ordinary skill in the art would understand that the residue on ignition, or results therefrom, should not be used to argue that the product of that method was essentially a single molecular species. In other words, this test could not confirm the presence of, or lack of, the harmful polyene/endotoxin contaminants addressed by the present invention.

6. The stated intent of the patent of Michel is to "provide an improved process for the purification and crystallization of amphotericin B". As indicated above, the process described by Michel uses methanol, dimethylformamide and acid to solubilize antibiotics, including amphotericin B. An analysis of the data provided indicates that the process resulted in improved removal of insoluble content (2.5% by the previous method) to 0.1% remaining, an improvement that corresponded with a yield of 97%.

7. The purity of the resulting amphotericin B (or other antibiotic) product was not directly addressed: the measurement of "residue on ignition" would not distinguish purity of the amphotericin. Nor would it distinguish between amphotericin B and other carbon-based compounds (other polyenes, other antibiotics). It would only provide a measure of the amount of insoluble, inorganic material in the final product.

8. Also, there was no comparison between 'residue after ignition' of the starting material and final product after crystallization in Michel's 4-

solvent system. Thus, there is no direct means to determine what extent of 'purification' might have occurred, if any. Further, there is no direct comparison of the composition of the final soluble product obtained from the process of Michel and the process of Dutcher. Therefore, there is also no valid means to compare the difference in the purities of amphotericin B between the two processes. It should be emphasized that 'residue after ignition' only indicates the amount of insoluble materials; it does not indicate the relative purity of amphotericin B in the final product relative to other soluble antibiotics and other classes of soluble compounds.

9. The improvement from the process of Michel (as claimed by Michel) is one of more consistent crystallization of product. There was no direct assessment of the composition of organic material or the relative purities of the individual components.

10. The present application of Cleary et al describes a process in which high performance liquid chromatography is used to obtain a final amphotericin B product (AmBHP) with a purity of 96% or better and with a level of impurities of no more than 4%. Data in the following table and the accompanying figure demonstrate the improvement in the process of amphotericin B purification obtained by the use of the process of Cleary et al compared to the method of Michel. These data also confirm the contention stated above (and in previous responses) that the method from which Michel

inferred purity of the amphotericin B is not accurate. The method of Michel neither changed the composition nor the relative abundance of any of the individual components in the antibiotic (amphotericin B).

Compound	Process	Apparent Purity
Amphotericin B, USP grade		90.4%
Amphotericin B	Michel	91.0%
AmBHP	Cleary et al	96.7%

11. In view of the above, it is respectfully submitted that the process described in Michel et al. would not result in a product having the claimed purity levels.

12. Tang, US '375, is also relied upon by the outstanding Office Action in connection with purification issues, methanolic suspension of antibiotics (including, amphotericin B) and bacteria. Tang discloses a method of purifying amphotericin B with an ion exchange column, removing gram positive and gram negative bacteria. From a review of this reference, one of ordinary skill in the art would understand that the disclosed ion exchange column process would not result in the features claimed. In other words, this

purification step should not result in a composition with the claimed purity characteristics. On the basis of Michel's terminology, the process of Tang is one of decontamination, not purification.

13. The Tang patent further does not address separation of amphotericin B from other polyene antibiotics. Additionally, there is no statement of purity of amphotericin B relative to other antibiotics or methanol soluble compounds.

14. Thus the method of Tang addresses a fundamentally different process than removal of polyenes and other soluble compounds that are present as impurities from amphotericin B (as addressed by the process of Cleary et al). Bacteria (and fragments thereof) are macromolecular structures (whole organisms or parts thereof) that are not soluble in the methanol used as solvent. In contrast, amphotericin B, other polyenes and, in fact, any other compound soluble in methanol are not separated by the ion exchange process, and Tang makes no claims to that effect.

15. In view of the above, it is respectfully submitted that the process described in Tang would not result in a product having the claimed purity levels.

16. The undersigned declares further that all statements made herein of his knowledge are true and that all statements made on information and belief are believed to be true; and further that these

statements were made with the knowledge that wilful false statements and the like so made are punishable by fine or imprisonment, or both, under section 1001 of Title 18 of United States Code and that such wilful false statements may jeopardize the validity of the application or any patent issuing thereon.

Signed this 6th day of February 2009.



Robert E. Kramer  
Robert E. Kramer

Figure:

Comparison of the abilities of the procedure of Michel and the procedure of Cleary et al to improve the purity of USP grade amphotericin B.

